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Messenger RNA prevalence in sea urchin embryos measured with cloned cDNAs

(RNA titration/cDNA clone library/colony hybridization)

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ABSTRACT **mRNA prevalence during sea urchin development was measured by treating cDNA clone colonies with labeled cDNAs transcribed from unfertilized egg and embryo poly(A)-RNAs. The number of cytoplasmic transcripts per embryo complementary to several clones was determined independently by titration with poly(A)-RNA in solution, and the amount of cDNA bound to these clones in colony hybridizations was shown to be proportional to the concentration of the respective poly(A)-RNAs in the embryo cytoplasm. At the gastrula stage, the most prevalent mRNA species occur in about 10^6 molecules per embryo. If all cells were equivalent, this would be a few hundred molecules per cell. By pluteus stage, the prevalence of some sequences has increased more than 10-fold. Most, though not all, sequences prevalent in later embryos are also present in the maternal RNA of the unfertilized egg. For most poly(A)-RNA sequences, the prevalence levels determined during oogenesis are maintained through the pluteus stage, whereas a minority of sequences display sharp stage-specific changes in representation during development.**

At the 600-cell gastrula stage, the sea urchin embryo contains about 10^8 polysomal mRNA molecules. A major fraction of the mRNA mass consists of moderately prevalent messages, whereas the remainder belongs to a complex sequence class composed of over 10^4 individual species, each represented in about 1000 copies per embryo (1, 2). The existence of these broad mRNA prevalence classes in the sea urchin embryo has been demonstrated by hybridization experiments carried out with single-copy (3, 4) and cDNA tracers (refs. 5–7 and unpublished data) and with cloned probes (8). However, the actual distribution of mRNA prevalence classes in the message population has not been determined, and no information is available on changes in the structure of the message population during differentiation of the embryo. To approach these issues, we measured the approximate prevalence of many different messages during embryonic development by means of colony hybridization of cDNA clone libraries with cDNAs transcribed from the poly(A)-RNA of unfertilized eggs and of gastrula and pluteus stage embryos. From this analysis we derive the number of cytoplasmic poly(A)-RNA sequences per embryo that are present at given prevalence levels. We encountered several examples of sequences expressed in a stage-specific manner. However, our results show that the sequence concentration of most cytoplasmic poly(A)-RNA sequences does not change significantly during development, and we believe that these observations reflect certain general properties of the regulatory systems operating during sea urchin development.

MATERIALS AND METHODS

cDNA Clone Libraries. Hybrids between oligo(dT)-primed cDNA and cytoplasmic poly(A)-RNA of gastrula and pluteus stage embryos were cloned according to Zain *et al.* (9) into plasmid pBR322 cut at *Bam*HI and *Eco*RI sites. Transformation by the procedure of Kushner (10) yielded 2000–3000 colonies per μg of hybrid; 500 gastrula and 1400 pluteus cDNA clones were used in these experiments. The average length of cDNA inserts, including poly(dA·dT) tails, was about 740 nucleotides (nt) ($n = 52$). According to genome blots, synthesis and turnover kinetic studies, and RNA gel blots (unpublished data), essentially all of the clones represent distinct sequences except for multiple occurrences of several very highly prevalent sequences (see below).

Colony Hybridization. Replicas of library microtiter plates were imprinted, grown, and amplified on Millipore nitrocellulose filters. After alkali denaturation, the filters were neutralized, then baked under reduced pressure at 80°C. For screening (11), the filters were first washed in 10× Denhardt's solution (Denhardt's solution is 0.2% bovine serum albumin/0.2% Ficoll/0.2% polyvinylpyrollidone/0.2% $NaDodSO_4$) for 2–4 hr at 68°C and then prehybridized at 68°C for 4 hr in 5 ml of SET solution (0.15 M NaCl/2.5 mM EDTA/30 mM Tris·HCl, pH 8.0), 25 mM phosphate buffer, 0.22 mM sodium pyrophosphate, 5× Denhardt's solution, 50 μg of sheared calf thymus DNA per ml, and 50 μg of poly(rA) per ml. The filters were then treated with $2.5–5 \times 10^7$ cpm of [^{32}P]cDNA of specific activity $\approx 2 \times 10^8$ cpm/μg in 5 ml per filter of the same solution for 24 hr at 68°C. After hybridization, the filters were washed at 68°C as follows: two washes of several hundred milliliters for 30 min each in SET solution/1× Denhardt's solution/25 mM phosphate buffer/0.22 mM sodium pyrophosphate and two further washes for 30 min each in 0.1× SET solution/25 mM phosphate buffer/0.22 mM sodium pyrophosphate/0.1% $NaDodSO_4$. They were then dried and exposed to preflashed x-ray film in the presence of a DuPont "lightning" intensifying screen.

Titration of cDNA Clones with Egg, Gastrula, or Pluteus Poly(A)-RNA. Restriction fragments containing cDNA inserts plus short flanking plasmid sequences were iodinated; the strands were separated; and the specific activity was determined (8, 12). Titrations were done as described (8) except that RNA·DNA hybrids were measured by digestion with nuclease S1 followed by binding to Whatman DE81 filters.

RESULTS

Quantitative Estimation of Transcript Prevalence from Intensity of Colony Hybridization. When cDNA clone libraries are grown on nitrocellulose filters and hybridized with radioactive embryo cDNA, some clones react extensively and others do not. A typical example is shown in Fig. 1*A*, which depicts an autoradiograph of a filter containing 90 randomly selected gastrula cDNA clones that had been hybridized with [^{32}P]cDNA transcribed from gastrula cytoplasmic poly(A)-

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Abbreviation: nt, nucleotide(s).

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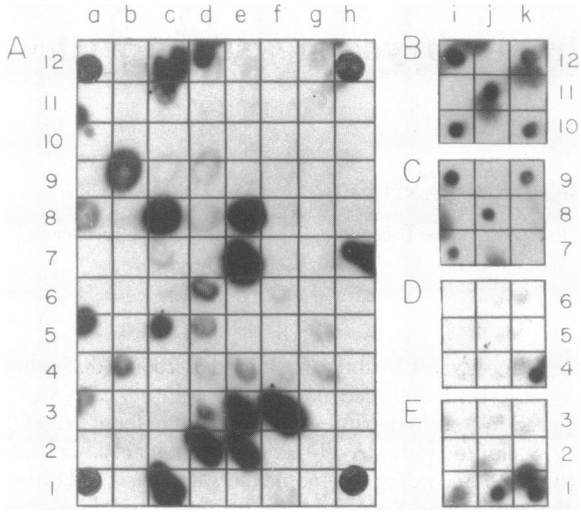

FIG. 1. Autoradiographs of cloned cDNA library screened with [^{32}P]cDNA. (*A*) Ninety gastrula cDNA clones were chosen at random and treated with [^{32}P]cDNA transcribed from gastrula cytoplasmic poly(A)-RNA. Clones studied in more detail below include SpG6, at position a8; SpG20, at position b10; and SpG30, at position c8. A colony containing pBR322 is at position c7. (*B–E*) Autoradiographs of selected subregions from larger filters. Five pluteus cDNA clones, previously observed to be represented by relatively prevalent pluteus cDNA species, were treated with [^{32}P]cDNA transcribed from pluteus poly(A)-RNA (*B*) or from adult intestine poly(A)-RNA (*C*). The colony at position k10 (*B*), clone SpP339, reacts with pluteus cDNA but not with intestine cDNA [position k7 (*C*)]. Nine gastrula cDNA clones, previously observed to hybridize with only small amounts of pluteus cDNA, were treated with [^{32}P]cDNA transcribed from total egg poly(A)-RNA (*D*) and from gastrula cytoplasmic poly(A)-RNA (*E*). The colonies at positions i4 (SpP16) and j4 (SpP17) in *D* react much more with gastrula poly(A)-RNA than with egg cDNA, as seen in *E*, positions i1 and j1.

RNA. The extent of reaction observed is a reproducible characteristic of each clone and is not simply a function of the cloned insert length. The average lengths of the cloned cDNA [including poly(dA·dT) tails] in seven of the least reactive clones was 952 nt; in 10 of the most reactive clones, it was 820 nt. When treated with cDNA transcribed from poly(A)-RNA of unfertilized eggs and from the cytoplasmic poly(A)-RNA of gastrula or pluteus stage embryos or adult sea urchin intestine, several clones displayed obvious stage-specific differences. Examples of such clones are shown in Fig. 1 *B–E*. These autoradiographs include a clone, SpP339, that reacts intensely with pluteus cDNA (Fig. 1*B*, k10) but not at all with intestine cDNA (Fig. 1*C*, k7) and two clones, SpP17 and SpP16, that react with gastrula cDNA (Fig. 1*E*, i1 and j1) but not with egg cDNA (Fig. 1*D*, i4 and j4).

Detailed information on the prevalence of a large number of individual cDNA species during development is potentially available in experiments such as that illustrated in Fig. 1. Several authors have used this method to provide useful qualitative descriptions of changes in sequence representation—e.g., during development of a slime mold (13) and of *Xenopus* embryos (14). The value of the approach would be enhanced if the amount of cDNA hybridizing to each colony could be simply related to the number of the corresponding transcripts in the cytoplasmic poly(A)-RNA. The following experiment demonstrates such a proportionality. The insert DNA in each colony is present in large sequence excess over the cDNA molecules, and our experience shows that variations in the growth of the individual clones are unimportant.

Three gastrula cDNA clones that react with very different amounts of [^{32}P]cDNA were chosen, and the quantity of poly(A)-RNA complementary to each was independently measured by the titration method (8, 12). The clones used for this experiment, SpG6, SpG20, and SpG30, are included in the screen illustrated in Fig. 1*A*. Reaction of SpG20 with gastrula [^{32}P]cDNA is barely observable, whereas SpG6 reacts to a moderate extent and SpG30 reacts to a relatively large extent. In Fig. 2, the measurements of transcript prevalence in egg, gastrula, and pluteus poly(A)-RNAs are shown for the separated strands of each of these three clones. Expression of the SpG20 sequence is regulated during development because these transcripts are observed only in gastrula stage embryos (Fig. 2*A*). The SpG6 and SpG30 sequences are expressed throughout

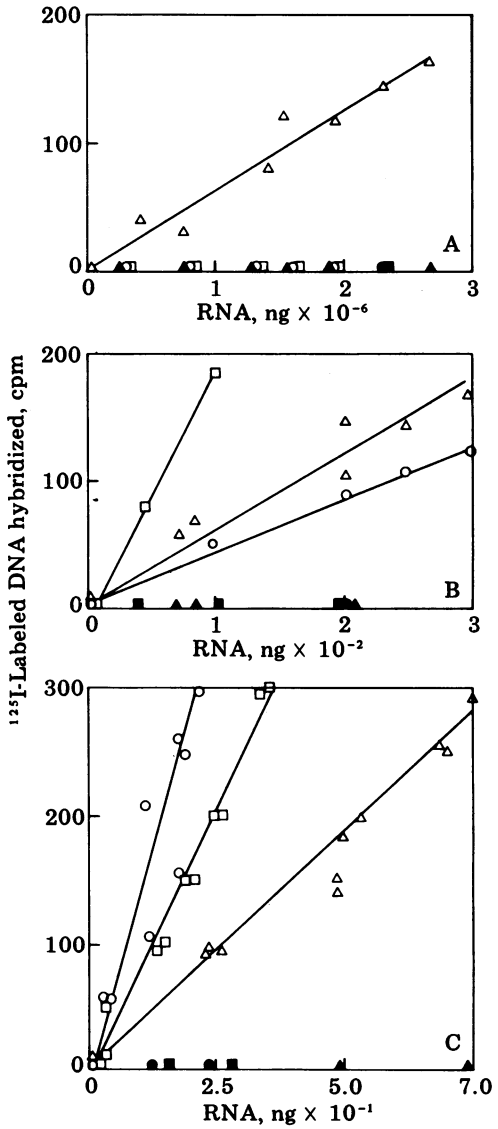

FIG. 2. Titration of cDNA clones with egg and embryo poly(A)-RNAs. Complementary strands (open and closed symbols) were incubated separately with the indicated amounts of unfertilized egg poly(A)-RNA (○ and ●) or with gastrula (△ and ▲) or pluteus (□ and ■) cytoplasmic poly(A)-RNA. Reactions were carried out to ≥10× $C_0t_{1/2}$ (moles of nt per liter × sec necessary for 50% hybridization), calculated with respect to the labeled DNA, which was present in sequence excess. The RNA·DNA hybrid formed during the reaction was measured by nuclease S1 resistance and was shown to be sensitive to RNase treatment at low salt concentration. The specific activities of the ^{125}I-labeled DNA tracers and the amounts used in each reaction were: (*A*) SpG20, 2.5 × 10^7 cpm/μg, 3000 cpm; (*B*) SpG6, 1.0 × 10^7 cpm/μg, 1025 cpm; (*C*) SpG30, 1.5 × 10^7 cpm/μg, 2500 cpm. The prevalence of the RNA transcripts was calculated from the slopes of the titration curves as described in the legend to Table 1. cDNA clone SpG20 contains an insert of 1050 nt, cDNA clone SpG6 contains an insert of 820 nt, and cDNA clone SpG30 contains an insert of 670 nt.

Table 1. Transcript prevalence for three cDNA clones measured by titration with poly(A)-RNA

Poly(A)-RNA	SpG6 transcripts		SpG20 transcripts		SpG30 transcripts	
	Per embryo	Average per cell	Per embryo	Average per cell	Per embryo	Average per cell
Egg	7,600	7600	<50	<50	450,000	450,000
Gastrula	11,000	18	330	0.5	110,000	190
Pluteus	23,000	15	<50	<0.03	340,000	220

Data are from Fig. 2. The number of transcripts (n) per embryo is calculated as follows (8): $n = fM/LS$, in which f is the slope of the titration curve (cpm ng^{-1}), M is the mass of poly(A)-RNA per embryo (ng), S is the specific activity of the ^{125}I-labeled DNA tracer used (cpm ng^{-1}), and L is the mass (ng) of the length of poly(A)-RNA hybridized after nuclease S1 treatment (i.e., the length of the cDNA inserts in the probe fragments). Values for S and L are given in the legend to Fig. 2. M is taken as 0.05 ng/embryo at all stages ($\approx 5 \times 10^7$ molecules of 2000 nt average length) based on Wilt (6) and data reviewed in ref. 1. We have assumed a 100-nt poly(dA·dT) tail. There are 600 cells in the gastrula embryo and about 1500 cells in the pluteus stage embryo.

development, though at somewhat different levels. Transcript prevalences for the various stages measured are listed in Table 1. In the cytoplasm of the 600-cell gastrula embryo there are about 330 molecules of the SpG20 transcript (Fig. 2*A*), about 11,000 molecules of the SpG6 transcript (Fig. 2*B*), and about 110,000 molecules of the SpG30 transcript (Fig. 2*C*). Only one strand of each insert is represented in any of the RNAs tested, as expected from earlier results (8).

To measure the amount of [^{32}P]cDNA hybridized by SpG6, SpG20, and SpG30 in a colony screen, we determined the radioactivity of the areas of the filter paper containing these colonies by liquid scintillation. In Fig. 3 the hybridized cpm (ordinate) are plotted against the gastrula poly(A)-RNA prevalence values from Table 1 (abscissa). For these three randomly chosen clones the extent of colony cDNA hybridization can be seen to be quantitatively related to the abundance of the complementary cellular transcripts. This experiment has been

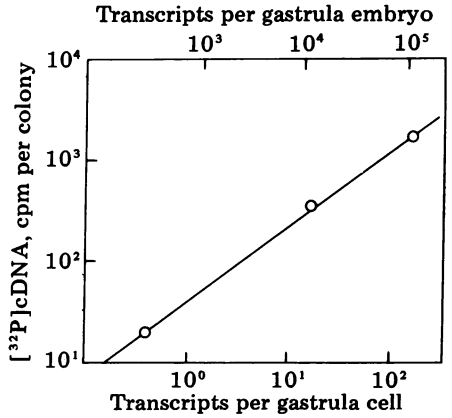

FIG. 3. Relationship between [^{32}P]cDNA colony hybridization and number of transcripts per embryo. The amount of [^{32}P]cDNA hybridized by colonies of SpG20, SpG6, and SpG30 (ordinate) is expressed as a function of gastrula transcript prevalence (abscissa) for these sequences. The amount of [^{32}P]cDNA used was 2.5×10^7 cpm and there is ≥1 ng of insert DNA per colony. Therefore, the insert DNA is present in significant excess over the homologous cDNA transcripts. The slope of the curve shown is 0.64, indicating that the amount of radioactivity hybridized is not directly proportional to the transcript prevalence. The slopes varied from about 0.2 (pluteus experiment) to about 0.6 [gastrula experiment (e.g., that shown)], probably depending on the cDNA preparation used. Background [^{32}P]cDNA (35 cpm) bound to colonies containing only pBR322 has been subtracted.

repeated many times with similar results, though the amount of counts hybridized varies according to the specific activity and amount of cDNA tracer used. By including SpG6, SpG20 (only in gastrula screens), and SpG30 as well as pBR322 background controls on each filter, an internal standard curve similar to that shown in Fig. 3 was generated for each experiment. From the amounts of [^{32}P]cDNA hybridized to any given cDNA clone colony it was thus possible to calculate the approximate number of molecules of complementary transcript in the original poly(A)-RNA by reference to the standard curve. This procedure is particularly useful for comparing the representation of clones in different RNAs because the prevalences calculated are independent of the amounts, the reactivities, or the specific activities of the particular cDNAs used. Independent estimates of steady-state transcript prevalence from comparisons of synthesis and decay rates have in every case agreed within a factor of 2 with the transcript prevalence established by colony screening.

Fig. 3 indicates that the sensitivity of the calibrated screening procedure may be extended down to a very low level of cytoplasmic expression if sufficient [^{32}P]cDNA is used and if background is kept low (i.e., about 30–50 cpm). Experiments in which rat hepatoma cDNA was used to screen the sea urchin cDNA library revealed no signals higher than the pBR322 background. Under our conditions, a sea urchin poly(A)-RNA sequence present at one copy per cell yields a signal equal in magnitude to the background. This is the expected result if such a sequence accounts for about 10^{-5} of the poly(A)-RNA and about 2.5×10^7 cpm of cDNA is used per experiment. Given the 5–10% efficiency measured for colony hybridization (not shown), it follows that 25–50 cpm of cDNA should be hybridized to a cDNA colony representing a poly(A)-RNA sequence present at one copy per cell. We conclude that even sequences belonging to the complex class of the embryo mRNA can be detected by this method, though quantitation is difficult at this level. However, the screening procedure is easily sensitive enough for estimation of transcript prevalence of 5–10 copies per cell upwards.

Poly(A)-RNA Prevalence Distribution in Gastrula and Pluteus Stage Embryos. The structure of the embryo cytoplasmic poly(A)-RNA populations was analyzed by screening randomly selected sets of 150 gastrula clones and 166 pluteus clones with the respective cDNAs. The fraction of clones reacting with [^{32}P]cDNAs of any given prevalence mirrors the mass fraction of poly(A)-RNA of that prevalence in the embryo. Results are shown in Fig. 4, expressed as a frequency histogram of the number of copies of each poly(A)-RNA species per embryo (or per average cell). Fig. 4 also shows estimates of the number of diverse poly(A)-RNA species per embryo (complexity) included in each of the arbitrary prevalence divisions shown.

A striking difference between gastrula and pluteus stage embryos is the presence in pluteus of a few sequences represented by $>10^3$ transcripts per average cell, some of which account for about 5% of the poly(A)-RNA, and the absence or scarcity of such prevalent transcripts in the gastrula. Were there more than a very small number of equally abundant gastrula sequences, they would easily have been detected in the sample of clones studied. Although no gastrula clones were observed in this prevalence range, Fig. 4 shows that in a screened sample of about the same size, 22 pluteus clones were found that belonged to high prevalence ($\geq 10^3$ copies per average cell) categories. There is, of course, uncertainty in the extrapolation used to calculate the highest pluteus prevalence (Fig. 4), but it seems inescapable that some sequences are far more concentrated in pluteus than in gastrula. This distinction is significant because the total amount of RNA (i.e., rRNA) in gastrula and pluteus stage embryos is about the same (1).

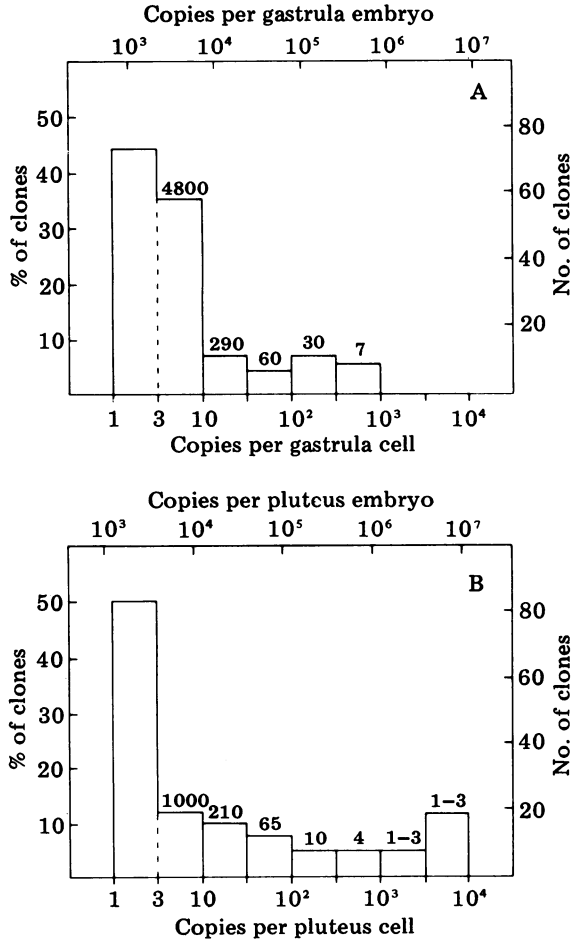

FIG. 4. Mass distribution of transcript prevalence in embryos calculated from cDNA clone hybridization. Random samples of 150 gastrula (*A*) and 166 pluteus (*B*) cDNA clones were screened with gastrula and pluteus [^{32}P]cDNAs. The relationship between [^{32}P]cDNA hybridized per colony and number of transcripts per embryo was determined for each filter by use of the internal standards. Data in Fig. 3 were used for the analysis of the 90 gastrula clones shown in Fig. 1*A*. The appearance in the autoradiograph of Fig. 1*A* of clones belonging to some of the prevalence categories in the histogram shown here can be observed in the following examples (clone position in Fig. 1*A*; copies per average cell): b4, 6; a10, 15; c1, 22; c11, 45; d3, 88; e2, 200; and e3, 450. The number of copies per average cell given on the lower abscissas are minimal estimates calculated on the basis that there are 600 cells per gastrula and 1500 cells per pluteus embryo and that all cells are assumed to be alike. The numerals above each bar in the histogram show the approximate number of diverse mRNA species per embryo calculated as $5 \times 10^7\, g/c$, in which g is the fraction of the clones falling in that category, c is the mean number of copies per embryo represented by that category, and 5×10^7 is the approximate number of poly(A)-RNA molecules in the cytoplasm of the embryo (Table 1). As indicated by the dashed line, we cannot determine the prevalence of very rare sequences with sufficient accuracy to permit a complexity calculation for these classes of transcript. Accurate estimates of the complexity of the rare mRNA classes are found in ref. 4. It would be expected statistically that some of the most prevalent pluteus poly(A)-RNA sequences will occur several times in the cDNA clone libraries. Analyses of 10 moderately and highly prevalent pluteus clones indicate that one sequence occurs three times, one twice, and five others once in the sample of 10. The 1400-clone library from which were randomly selected the 166 pluteus clones used in the experiment shown here probably contains all or most of the diverse sequences included in the prevalence classes from 100 copies per average cell upwards, but only about 10–15% of the complex class species (4).

About 40% of the gastrula poly(A)-RNA and 50% of the pluteus poly(A)-RNA belong to the complex sequence class, in which each RNA species in present in only one to a few copies per average cell. There are about 5×10^7 poly(A)-RNA molecules of average length 2000 nt per embryo at gastrula stage [about half of the polysomal mRNA is significantly polyadenylylated (1)], so that the number of complex class poly(A)-RNA molecules (mass) per gastrula cell is approximately 3×10^4. The fraction of complex class RNAs observed here is 2-fold higher than estimated earlier from single-copy tracer hybridization kinetics (1, 2) but it is consistent with a cDNA hybridization kinetic analysis (not shown), although previous analyses carried out by that method were interpreted differently (5, 7). One factor that may affect the comparison is that the single-copy DNA hybridizations were performed with polysomal RNA, whereas the present analysis (and the cDNA hybridization kinetic measurements) refer to cytoplasmic poly(A)-RNA.

Large Changes in Transcript Prevalence During Development Are Infrequent. Fig. 4 implies that most of the diverse genes represented in cytoplasmic poly(A)-RNAs produce only rare transcripts during development, whereas a minority displays the capacity to produce transcripts present at more than 10 copies per cell on the average. To test this directly, we screened a sample of 40 gastrula clones represented by rare gastrula poly(A)-RNAs ($<2 \times 10^3$ copies per embryo) and 40 pluteus clones represented by rare pluteus poly(A)-RNAs ($<5 \times 10^3$ copies per embryo) with [^{32}P]cDNAs transcribed from egg, gastrula, and pluteus poly(A)-RNA. This experiment revealed only 5 of the 80 clones that at any stage were represented by moderately prevalent rather than rare transcripts. Two of these five examples (SpP16 and SpP17) are illustrated in Fig. 1 *D* and *E* and, when most intensely expressed, all five fell into the $6–18 \times 10^3$ transcript per embryo class (i.e., 10–30 copies on the average per gastrula cell). This result suggests that many of the sequences in this prevalence class are represented by rarer transcripts at other stages (i.e., that they are subject to regulation). The sensitivity of this experiment is not sufficient to detect changes in level of expression within the domain of low transcript abundance—e.g., such as those demonstrated by the more sensitive titration method for SpG20 (Fig. 2*A*). However, the experiment shows directly that during development very few of the complex class sequences ever give rise to higher prevalence transcripts present at more than 10 copies per cell on the average.

A similar experiment was carried out with 40 more highly represented sequences ($>10^4–10^5$ copies per embryo) drawn

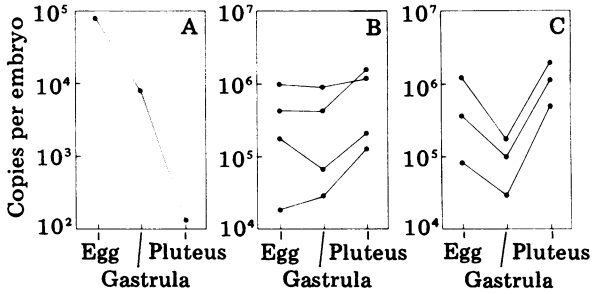

FIG. 5. Developmental change in representation of prevalent sequences. Twenty gastrula clones, represented by prevalent cytoplasmic poly(A)-RNAs from gastrula, and 20 pluteus clones, represented by prevalent cytoplasmic poly(A)-RNAs from pluteus, were screened with [^{32}P]cDNA transcribed from unfertilized egg poly(A)-RNA and with [^{32}P]cDNAs transcribed from cytoplasmic poly(A)-RNAs of gastrula and pluteus. Only a few representative examples are shown in each panel. The number of clones displaying each pattern is as follows. (*A*) Single example observed of a clone (SpG190) represented at high levels in the egg and very low levels, if at all, in pluteus. (*B*) Twenty-seven clones were represented at levels the same (within a factor of 2) in egg and gastrula, but at 2–4 times higher levels in pluteus than in gastrula. This increase would preserve equal transcript concentrations per cell. (*C*) Twelve clones were represented at least 10 times more frequently in pluteus than in gastrula.

from gastrula and pluteus libraries. Transcript prevalence was measured for these 40 clones in all three poly(A)-RNAs. Fig. 5 illustrates the patterns observed and indicates the numbers of clones displaying each. Though many other patterns of expression are possible, all of the 40 clones fell into the three classes illustrated. One striking result is that none of the 40 embryo sequences in this sample is either absent or rare in egg poly(A)-RNA. Sequences that are relatively prevalent in late embryos and rare in egg RNA do occur, though infrequently. For example, in a different experiment not shown here a high prevalence pluteus stage clone, SpP294, was found to be undetectable in egg RNA, and this is also true of three clones represented at moderate levels in gastrula RNA—namely, SpG2 (data not shown) and the two clones shown in Fig. 1 *D* and *E*, SpP16 and SpP17. However, only a few similar cases have come to light in screening of over 1400 pluteus clones. In the large majority of cases, the high abundance of the prevalent late embryo sequences was evidently established during oogenesis because it is already apparent in the maternal RNA stored in the unfertilized egg. A second major result shown in Fig. 5*C* is that late in development the concentration of many prevalent and moderately prevalent mRNA species increases ≥10-fold relative to their concentration at earlier stages.

DISCUSSION

These experiments demonstrate that unfertilized sea urchin egg RNA includes most of the prevalent mRNA species found in the advanced embryo. Earlier studies showed that this is also true of most of the rare polysomal RNAs of gastrula and pluteus stages (4, 15). One possibility is that the same proteins are required in the early embryo as in later differentiated stages. Alternatively, the presence of maternal transcripts could in some way be necessary to program the first embryo nuclei to express the same sequences, as suggested earlier (16).

Two-dimensional electrophoretic analyses of newly synthesized embryo proteins indicated that a majority of the proteins whose synthesis is detectable in later embryos are also being synthesized at the earliest stages of development (17). We now see that these protein synthesis experiments probably refer to all or most of the mRNA species above the lowest prevalence classes because as many as 500–1000 diverse proteins can be resolved (17, 18). Comparison with Fig. 4 shows that, to account for this complexity, the corresponding sample of mRNAs must consist of essentially all of those that average ≥5–10 copies per gastrula cell. The spectrum of protein species translated *in vitro* from embryo poly(A)-RNA is almost identical with that synthesized *in vivo* (19). Therefore, there can be little doubt that cytoplasmic poly(A)-RNAs present in such low concentration as to average only 5–10 copies per cell are functional embryonic messages. Messages of this and greater prevalence include about 50–60% of the mass of the poly(A)-RNA and about 10% of its complexity. The remaining 40–50% of the poly(A)-RNA mass includes 90% of the complexity of the polysomal mRNA studied earlier (3, 4, 15).

There are clearly certain mRNA sequences that are regulated during development. According to Brandhorst (17) some changes in the visible protein synthesis pattern occur around gastrula stage, and by late pluteus stage there is an increase of about 20% in the number of prominent newly synthesized species. The samples of prevalent cDNA clones studied here is too small to permit an independent measurement of the overall frequency of large developmental changes because these are not common. However, it is likely that the marked increase in transcript prevalence often observed at pluteus stage (Fig. 5) accounts for some of the visible changes in protein synthesis patterns reported by Brandhorst (17). Another general class of changes seen at the polysomal RNA level is the virtual disappearance of many complex class species after cleavage (4). A cloned sequence called Sp88, which displays this behavior, has recently been described in detail (8), and among prevalent sequences, SpG190 (Fig. 5*A*) behaves similarly. Other sequences discussed in this paper attain detectable levels of expression only during embryogenesis—e.g., SpP16, SpP17 (Fig. 1 *D* and *E*), SpG20 (Fig. 2*A*), SpP294, and SpG2. The example of the SpG20 sequence that is not detectable in the egg even by the sensitive titration method (Table 1) shows that not every poly(A) sequence is stored in the maternal RNA.

Fig. 5 shows that the levels of expression of individual RNA sequences in the embryo vary by several orders of magnitude. The processes that determine the levels for each mRNA are obviously a fundamental aspect of gene regulation. By the gastrula stage, essentially all of the mRNAs in the embryo are newly synthesized rather than maternal (20). Measurements of the cytoplasmic entry and decay rates of a set of specific cloned sequences (J. Ellison and E. Davidson, unpublished data) show that highly prevalent cytoplasmic transcripts such as SpG30 decay many times more slowly than do less abundant sequences and that they also display higher rates of flow into the cytoplasm. Though the mechanisms by which transcript prevalence is established in oogenesis must be very different from those operating in the late embryo, when there are 1000 times more nuclei, the concentration of most RNA sequences is almost the same as in the mature egg. Many of the most prevalent sequences may be represented at a high level in egg RNA simply because these genes are expressed at a relatively high level whenever they are transcribed (2).

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